

The Utility of Ancestral and Derived Allele Sharing for Genome-Wide Inferences of Introgression

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Abstract

The past decade has ushered in a resurgence of studies highlighting the importance of introgression throughout the Tree of Life. Several methods exist for detecting and quantifying introgression on a genomic scale, yet the majority of these methods primarily utilize signals of derived allele sharing between donor and recipient populations. In this study, we exploit the fact that introgression will not only result in derived allele sharing but also the reintroduction of ancestral alleles to derive new estimators of the admixture proportion. Using coalescent simulations, we assess the performance of our new methods and the methods proposed in *Lopez Fang et al. 2022* to assess the utility of incorporating shared ancestral variation into genome-wide inferences of introgression. Using coalescent theory, simulations, and applying our methods to human and canid data, we find that methods incorporating ancestral allele sharing are comparable to their derived allele sharing counterparts, in turn providing researchers with the opportunity to utilize more of the genomic signature of introgression.

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1 Introduction

The field of evolutionary biology has seen a meteoric rise in the number of studies detecting and quantifying instances of introgression—the integration of foreign genomic blocks into a species’ genomic background—throughout the Eukaryotic Tree of Life (Dagilis et al., 2022) since the publishing of the draft Neanderthal genome in 2010 (Green et al., 2010). This is in large part due to the recent feasibility of next-generation sequencing technology, which has initiated a paradigm shift in how evolutionary biologists study introgression from comparing sequence similarity at some genetic markers between species to analyzing the whole genome via statistical methods—e.g., *Patterson’s D*—to make inferences of introgression by using site patterns as a proxy for genealogical relationships (Dagilis et al., 2022; Durand et al., 2011; Edelman & Mallet, 2021; Green et al., 2010). Recent years have ushered in an influx of studies demonstrating that introgression is not only more common than originally thought, but can also provide the raw genetic materials necessary for local adaptations (Hedrick, 2013; Huerta-Sánchez et al., 2014; Jagoda et al., 2022; Jones et al., 2018; Suarez-Gonzalez et al., 2018; Zhang et al., 2021), adaptive radiations (Malinsky et al., 2018; Meier et al., 2017; Pease et al., 2016; Richards & Martin, 2017; Stankowski & Streisfeld, 2015), and speciation (Bierne et al., 2013; Nelson et al., 2021; Rheindt & Edwards, 2011). However, even with keystone studies demonstrating that the evolutionary outcomes of introgression are diverse, it is challenging to assess its significance in evolution because the frequency of introgression across the Tree of Life is still unclear (Dagilis et al., 2022; Edelman & Mallet, 2021).

The most popular statistical methods for making genome-wide inferences about introgression exploit asymmetries in observed site patterns (Dagilis et al., 2022). Statistical tests for site pattern asymmetries count the number of segregating sites that have a configuration of shared derived and ancestral alleles which are informative of introgression in a set of taxa. One example of these tests is *Patterson’s D* (Equation 1), where two configurations of derived allele sharing site patterns are used to test for the presence of introgression (Durand et al., 2011; Green et al., 2010). Genome-wide tests of introgression based on observed site patterns are relatively straight-forward to implement, computationally efficient to calculate, and provide a unified approach for detecting and quantifying introgression across the Tree of Life as they can be applied to both phylogenetic and population genetic data sets. Due to the utility of site pattern-based metrics of introgression, recent years have seen a rapid proliferation of site pattern-based statistics designed to detect and quantify genome-

31 wide levels of introgression (Durand et al., 2011; Hamlin et al., 2020; Lopez Fang et al., 2022;
32 Martin et al., 2014; Pease & Hahn, 2015; Pfeifer & Kapan, 2019). However, until recently existing
33 methods have strictly relied on the idea of derived allele sharing—where the donor and recipient
34 populations both harbor the same derived allele—which only captures a small portion of the total
35 genomic signature produced by introgression (Lopez Fang et al., 2022). Notably, *Lopez Fang et al.*
36 *2022* recently proposed the $D+$ statistic, which leverages both the sharing of derived and ancestral
37 alleles between donor and recipient populations to detect genomic windows harboring introgressed
38 segments in the genome.

39 In this study we aim to assess if ancestral allele sharing site patterns are informative about
40 introgression on a broader genomic scale. We first propose novel metrics to quantify the proportion
41 of introgression that utilize ancestral allele sharing site patterns. Using coalescent simulations we
42 assessed the performance of our newly proposed metrics and the metrics to detect introgression
43 proposed by *Lopez Fang et al. 2022*. Lastly, we assessed the performance in empirical data by ap-
44 plying all the introgression metrics to publicly available human and canid data sets (1000 Genomes
45 Project Consortium et al., 2015; Freedman et al., 2014). We find that in both simulated and empir-
46 ical data, all introgression metrics perform comparably, allowing researchers the opportunity to use
47 multiple lines of evidence across more segregating sites throughout the genome to make inferences
48 about introgression.

49 2 New Approaches

50 In practice, most site pattern-based metrics of introgression assume a four-taxon tree—Newick
51 format: $((P1, P2), P3), O$; site pattern format: $(P1's\ allelic\ state, P2's\ allelic\ state, P3's\ allelic$
52 $state, O's\ allelic\ state)$ —where $P1$ and $P2$ represent potential recipients of introgression from the
53 donor population $P3$, and O represents an outgroup used to polarize ancestral states, in which A
54 denotes the ancestral allele and B denotes the derived allele. Thus, the $ABBA$ site pattern indicates
55 that the derived allele is shared between $P2$ and $P3$, while the $BABA$ site pattern indicates that
56 the derived allele is shared between $P1$ and $P3$. *Patterson's D* (Equation 1) specifically tests for
57 patterns of derived allele sharing between donor and recipient populations and a significant excess
58 of either aforementioned site pattern is suggestive of introgression (Durand et al., 2011; Green et al.,
59 2010)

$$D(P1, P2, P3, O) = \frac{\sum_{i=1}^n C_{ABBA}(i) - C_{BABA}(i)}{\sum_{i=1}^n C_{ABBA}(i) + C_{BABA}(i)} \quad (1)$$

60 where $C_{ABBA}(i)$ and $C_{BABA}(i)$ are indicator variables that take a value of either zero or one condi-
 61 tioned on observing an *ABBA* or *BABA* site pattern at site i for all n sites where we have data for
 62 all four samples (Durand et al., 2011; Green et al., 2010). Due to its computational efficiency and
 63 ability to discriminate between coalescent histories of incomplete lineage sorting (ILS)—the process
 64 where two lineages fail to coalesce in their most recent common ancestral population—and intro-
 65 gression, *Patterson’s D* is the most widely used summary statistic to detect introgression (Dagilis
 66 et al., 2022). However, there is no theoretical or biological reason to believe that introgression will
 67 only result in the sharing of derived alleles. This is because introgressed genomic segments contain
 68 both new derived alleles and re-introduced ancestral alleles. Using this logic, *Lopez Fang et al.*
 69 *2022* derived the expected branch lengths where a single mutation would generate a *BAAA* and
 70 *ABAA* site pattern respectively, which represents the ancestral allele sharing analogs to the *ABBA*
 71 and *BABA* site patterns. Notably, the authors demonstrated that the expected difference in the
 72 number of *ABBA* and *BABA* sites is equivalent to the expected difference in the number of *BAAA*
 73 and *ABAA* sites in the instantaneous unidirectional admixture (IUA) model of introgression—see
 74 the Appendix for the model description—providing the motivation to construct D_{anc} (Equation 2),
 75 a statistic that leverages ancestral allele sharing to detect local introgressed regions

$$D_{anc}(P1, P2, P3, O) = \frac{\sum_{i=1}^n C_{BAAA}(i) - C_{ABAA}(i)}{\sum_{i=1}^n C_{BAAA}(i) + C_{ABAA}(i)} \quad (2)$$

76 where $C_{BAAA}(i)$ and $C_{ABAA}(i)$ are indicator variables that take a value of either zero or one
 77 conditioned on observing a *BAAA* or *ABAA* site pattern at site i for all n sites where we have
 78 data for all four samples. They also defined $D+$, a statistic (Equation 3) which leverages both the
 79 sharing of derived and ancestral alleles to strengthen inferences for detecting locally introgressed
 80 genomic regions

$$D+(P1, P2, P3, O) = \frac{(\sum_{i=1}^n C_{ABBA}(i) - C_{BABA}(i)) + (\sum_{i=1}^n C_{BAAA}(i) - C_{ABAA}(i))}{(\sum_{i=1}^n C_{ABBA}(i) + C_{BABA}(i)) + (\sum_{i=1}^n C_{BAAA}(i) + C_{ABAA}(i))} \quad (3)$$

81 and found that $D+$ was a more reliable statistic than D to detect introgression in small genomic
 82 windows (Lopez Fang et al., 2022). As researchers are also interested in quantifying the amount

83 of introgression, here we construct estimators to infer the fraction of the genome shared through
84 introgression—hereafter referred to as the admixture proportion. One approach to infer the ad-
85 mixture proportion is to assess the ratio of observed differences in allele sharing between the donor
86 and recipient populations—i.e., the $P3$ and $P2$ populations respectively—in the numerator and
87 the expected differences assuming the entire genome was introgressed—i.e., complete homogeniza-
88 tion of allele sharing—from $P3$ to $P2$ in the denominator (Martin et al., 2014). This approach
89 (Equation 4) was first described only in terms of derived allele sharing between recipient and donor
90 populations in Martin et al. 2015 as an alternative to the original estimator of the admixture
91 proportion described in Durand et al. 2011 where two pseudo-haploid genomes are created by
92 randomly sub-sampling chromosomes from the $P3$ individual. In the simplest case of only having
93 four whole-genome alignments—which are monoploid—this pseudo-haploid approach is not possi-
94 ble, so Martin et al. 2015 proposed to use the sampled $P3$ sequence twice in the denominator to
95 circumvent the need for creating pseudo-haploid genomes. The original estimator of the admixture
96 proportion from Martin et al. 2015 is f_{hom} (Equation 4)

$$S(P1, P2, P3, O) = \sum_{i=1}^n C_{ABBA}(i) - C_{BABA}(i) \quad (4a)$$

$$f_{hom}(P1, P2, P3, O) = \frac{S(P1, P2, P3, O)}{S(P1, P3, P3, O)} \quad (4b)$$

97 where homogenization is accomplished by replacing $P2$ with $P3$ in the assumed underlying species
98 tree—i.e., in Newick format $((P1, P3), P3), O$ —when computing the denominator. It should be
99 noted that when written in terms of derived allele frequencies and in the most simplest case of only
100 having four whole-genome sequences, f_{hom} is equivalent to $F4$ -ratio (Patterson et al., 2012). Given
101 that patterns of ancestral allele sharing between donor and recipient populations mirror those of
102 their derived allele sharing counterparts we can re-derive f_{hom} in terms of $BAAA$ and $ABAA$ site
103 patterns resulting in f_{anc} (Equation 5).

$$S_{anc}(P1, P2, P3, O) = \sum_{i=1}^n C_{BAAA}(i) - C_{ABAA}(i) \quad (5a)$$

$$f_{anc}(P1, P2, P3, O) = \frac{S_{ANC}(P1, P2, P3, O)}{S_{ANC}(P1, P3, P3, O)} \quad (5b)$$

104 Lastly, given that introgressed segments are inherited in blocks consisting of both new derived
105 alleles and re-introduced ancestral alleles one can formulate quantification metrics that consider all
106 possible site patterns that represent allele sharing between recipient and donor populations; such
107 as $f+$ (Equation 6).

$$S + (P1, P2, P3, O) = \left(\sum_{i=1}^n C_{ABBA}(i) - C_{BABA}(i) \right) + \left(\sum_{i=1}^n C_{BAAA}(i) - C_{ABAA}(i) \right) \quad (6a)$$

$$f + (P1, P2, P3, O) = \frac{S + (P1, P2, P3, O)}{S + (P1, P3, P3, O)} \quad (6b)$$

108 Note that all coalescent based expectations and equations written in terms of derived allele fre-
109 quencies can be found in the Appendix.

110 3 Results

111 3.1 Ancestral & Derived Allele Sharing Site Pattern Differences Are Equivalent 112 Across All Admixture Proportions

113 We first validated the theoretical result proposed by *Lopez Fang et al. 2022* that the ($ABBA -$
114 $BABA$) and ($BAAA - ABAA$) site pattern differences are equivalent by simulating whole-genome
115 sequences under an IUA model of introgression using parameters that reflect the demographic
116 history of humans (Figure S1) using the coalescent simulator `msprime v1.1.1` (Baumdicker et
117 al., 2022). For each simulation replicate, we sample one chromosome from each population, and
118 computed the difference—i.e., ($ABBA - BABA$) - ($BAAA - ABAA$). We simulated 100 replicates
119 under the demographic model to generate a distribution of the difference. We calculated the mean
120 of the difference over the number of replicates and we take the absolute value of the mean—so that
121 the mean difference is always positive. We then test to see if the mean is statistically significantly
122 different from zero. For different values of the admixture proportion ranging from 0% to 50%,
123 we find that the difference of site pattern differences does not significantly differ from zero under
124 the IUA model (see Figure 1 and Table S4). This is also true in the case of a more complex
125 demographic history—e.g. such as the one in *Ragsdale and Gravel 2019*—or when we consider a
126 larger sample size ($n=100$), where the variance of the differences gets smaller (see Figure 1; Tables
127 S4-S6 and S19-S20). These results suggest that the theoretical expectation is robust to sampling

128 scheme, demographic history, and the amount of introgression, providing us with justification for
 129 constructing introgression metrics that incorporate ancestral allele sharing site patterns.

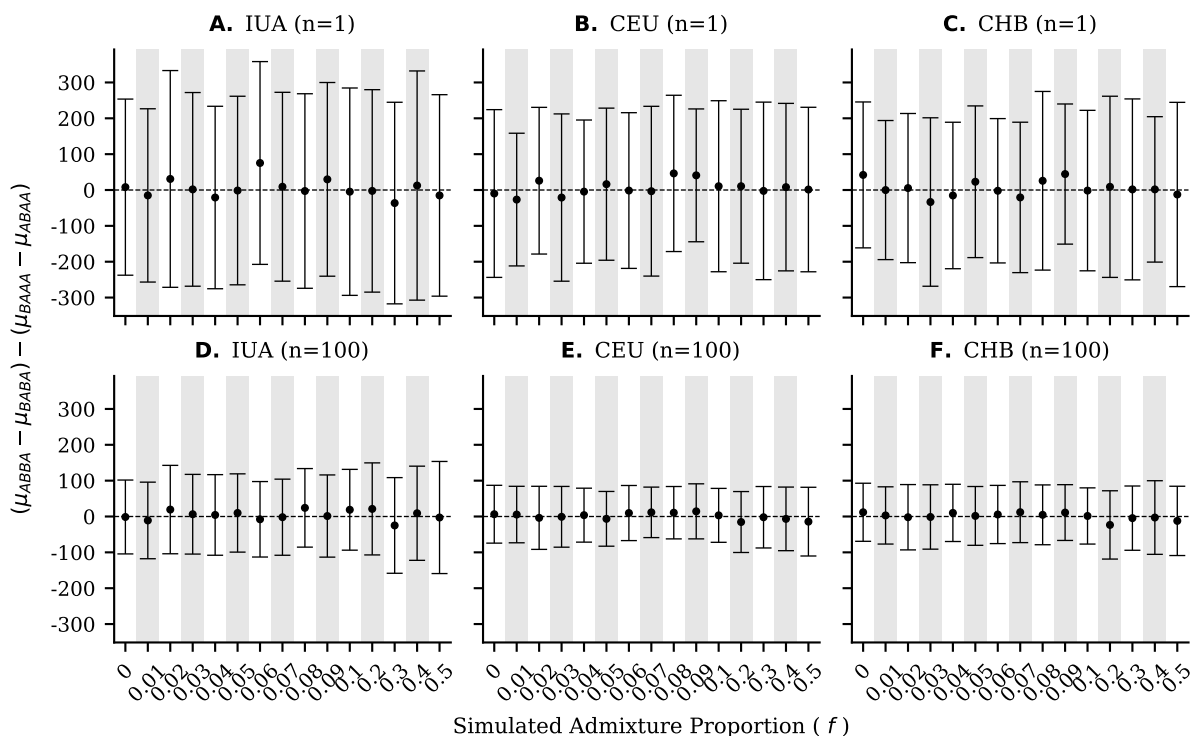


Figure 1: The mean (dots), standard deviation (error bars), and the theoretical expectation (dashed line and $y = 0$) for the difference of site pattern differences in simulations under an IUA model of introgression (panels **A** and **D**) and a more realistic model of human demography modified from *Ragsdale and Gravel 2019* (panels **B-C** and **E-F**) with a sampling scheme of $n = 1$ and $n = 100$ genomes from each of the potential recipient populations.

130 **3.2 The Power To Detect Introgression Is Comparable Across All Metrics**

131 Simulating under the IUA demographic model, we generated distributions of D , D_{anc} , and $D+$
 132 and subsequently computed the mean simulated value per admixture proportion. Table **S10** shows
 133 that the mean simulated values for these statistics match the values of D , D_{anc} and $D+$ calcu-
 134 lated using the theoretical expectations of the number of $ABBA$, $BABA$, $BAAA$, and $ABAA$ site
 135 patterns (Equations **A1-A3**). We next assessed how the power to correctly detect introgression in
 136 simulated data differed between D , D_{anc} , and $D+$. We define power as the number of replicates

137 that significantly differ from zero out of 100 total simulated replicates per admixture proportion; to
138 test for statistical significance we constructed 1000 bootstrapped genomes per simulation replicate
139 and calculate each introgression decision metric, which were used to generate a z -distribution and
140 subsequent p -values. Like D , $D+$ and D_{anc} are able to correctly identify instances of introgression
141 and we observe that the power to correctly identify instances of introgression is largely consistent
142 across all metrics (Figure 2; Tables S7-S9). Notably, in simulations with no introgression (i.e.,
143 when $f = 0$) $D+$ and D_{anc} either always incorrectly detect introgression at the same rate or lower
144 rate as D , suggesting that on average $D+$ and D_{anc} have a lower false positive rate (FPR) than
145 D . Under the IUA model of introgression D and $D+$ are always able to correctly identify intro-
146 gression at comparable rates and outperform D_{anc} . For more realistic demographic histories, on
147 average D slightly outperforms $D+$ for smaller amounts of introgression while both statistics tend
148 to outperform D_{anc} (Tables S8-S9). Additionally, the power to correctly detect introgression always
149 increased when the sample size increases in all models explored (Figure 2; Tables S7-S9).

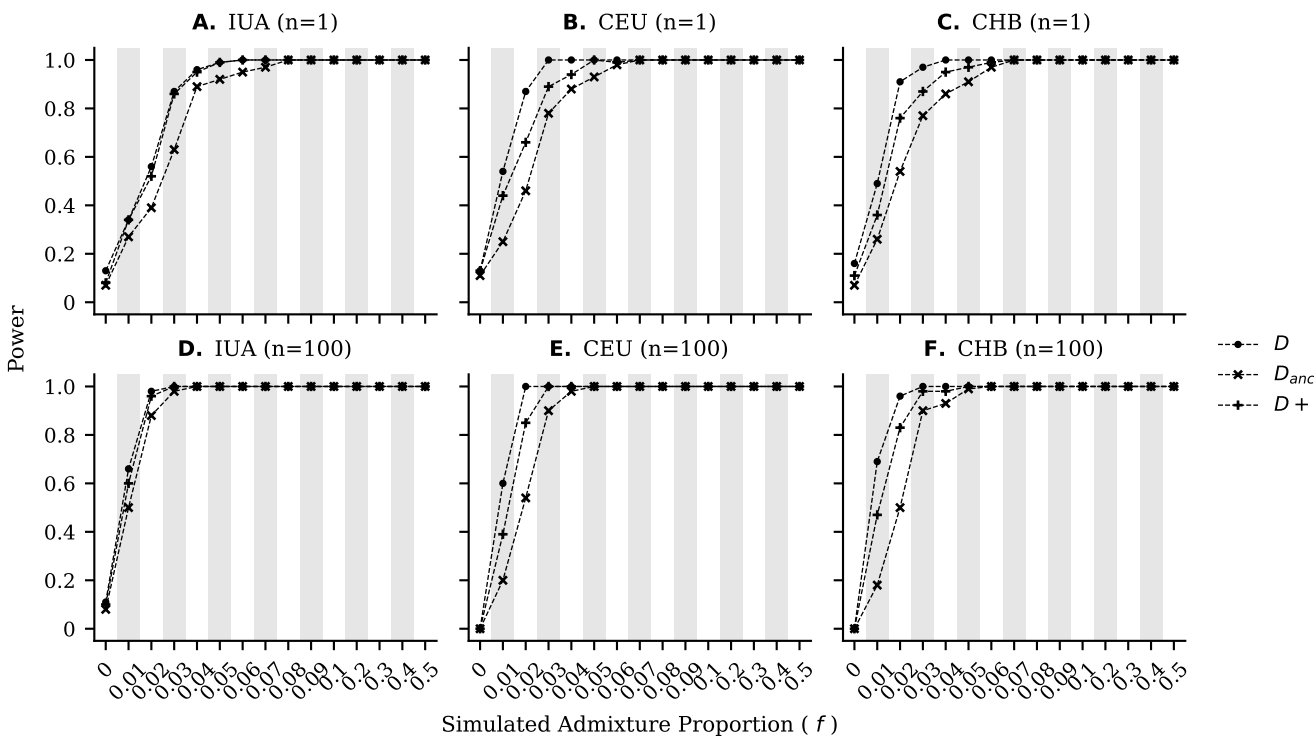


Figure 2: The power to correctly detect introgression in simulations under an IUA model of introgression (panels **A** and **D**) and a more realistic model of human demography modified from *Ragsdale and Gravel 2019* (panels **B-C** and **E-F**) with a sampling scheme of $n = 1$ and $n = 100$ genomes from each of the potential recipient populations. Note that the power at $f = 0$ corresponds to the false positive rate since no introgression has occurred.

150 3.3 The Power To Quantify Introgression Is Nearly Identical Across All Metrics

151 Simulating under the IUA model with varying admixture proportions from 0% to 50% we gen-
 152 erated distributions and computed the simulated mean values of f_{hom} , f_{anc} , and $f+$. Notably, the
 153 coalescent based expectations closely mirror the mean inferred admixture proportions across all
 154 simulations, providing evidence that f_{hom} , f_{anc} , and $f+$ are all accurate metrics for quantifying in-
 155 trogression given the underlying IUA model (Figure 3; Table S16). We next evaluated the power of
 156 f_{hom} , f_{anc} , and $f+$ to infer the true admixture proportion in simulated data under the assumption
 157 that a perfect estimator of the admixture proportion would infer the same value as the simulated ad-
 158 mixture proportion—i.e., $\hat{f} = f$. Under this assumption we calculated the root-mean-square-error

159 (*RMSE*) and mean-absolute-error (*MAE*) of each quantification metric for all simulated admixture
 160 proportions. Despite that f_{hom} , f_{anc} , and $f+$ all generally underestimate the true admixture propor-
 161 tion for our demographic models, the power to infer the admixture proportion is nearly identical
 162 across all metrics (Table S13-S15). In fact, Tables S16-S18 demonstrate that the mean inferred
 163 admixture proportions varied at most by 0.1% across all metrics for any given demographic model,
 164 sampling scheme, and simulated admixture proportion. However this is to be expected given that
 165 f_{hom} , f_{anc} , and $f+$ all have the same coalescent based expectation—i.e., $\frac{(f) \cdot (T_{P3} - T_{GF})}{T_{P3} + 2N}$ (see equations
 166 A7-A9 in the Appendix).

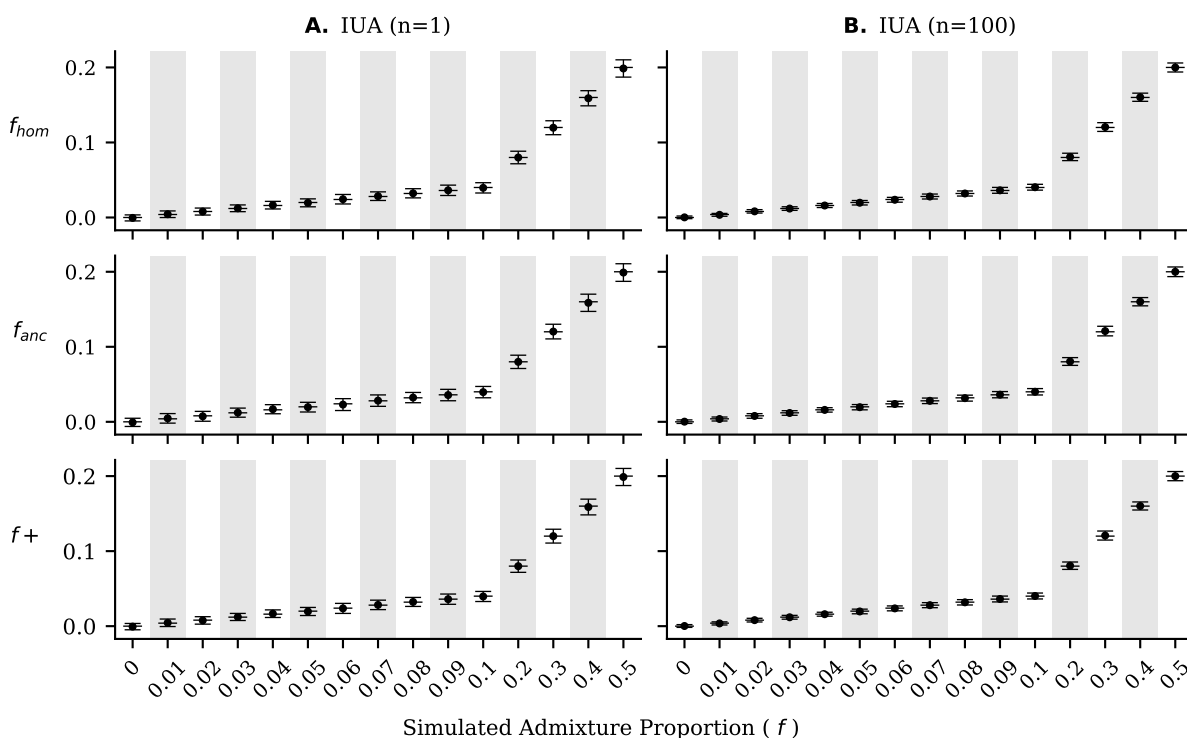


Figure 3: The mean (dots), standard deviation (error bars), and the theoretical expectation (solid horizontal lines) of f_{hom} , f_{anc} , and $f+$ in simulations under an IUA model of introgression with a sampling scheme of $n = 1$ (panel **A**) and $n= 100$ (panel **B**) genomes from each of the potential recipient populations.

167 3.4 All Introgression Metrics Perform Similarly In Human & Canid Data

168 Before calculating f_{hom} , f_{anc} , and $f+$ in empirical data, we wanted to investigate the behavior of
169 ancestral and derived allele sharing site patterns. We first calculated $ABBA$, $BABA$, $BAAA$, and
170 $ABAA$ using the modern-day humans in the 1000 Genomes Project (TGP) and the high-coverage
171 Altai Neanderthal genome (1000 Genomes Project Consortium et al., 2015; Prüfer et al., 2014).
172 We first wanted to test if the difference of site pattern differences significantly deviated from zero
173 by calculating site pattern counts for every non-African individual in the TGP and calculating the
174 mean difference of site pattern differences per chromosome for every population. Concordant with
175 our theoretical and simulated results, on average the mean difference of site pattern differences
176 per chromosome was within two standard deviations of the theoretical expectation of zero (Figure
177 4). However, it should be noted that not all chromosomes were within two standard deviations,
178 particularly, this was the case for chromosome 19 in every non-African population (Figure 4).

179 Additionally, we computed the distribution of the difference across the whole genome (Figure
180 5) to assess if the genomic mean difference of site pattern differences significantly differed from
181 zero in each population. After correcting for multiple comparisons, we found that only the CDX
182 population did not meet our theoretical expectation (Figure 5; Table S21). Next, we inferred the
183 Altai Neanderthal admixture proportion in each non-African individual and again concordant with
184 our theoretical and simulated results we find that all estimates of the admixture proportion are
185 concordant among trios with the mean f_{hom} , f_{anc} , and $f+$ values all being within one standard
186 deviation of one another within each non-African population (Figure 6; Table S22). As all the
187 statistics studied here can also be defined as functions of allele frequency (see Equations A4-A6
188 and A10-A12), we also used the derived allele population frequencies to infer the presence and
189 amount of introgression in every non-African population. Remarkably, we find that all D , D_{anc} ,
190 and $D+$ estimates are statistically significant after correcting for multiple comparisons and that
191 estimates of f_{hom} , f_{anc} , and $f+$ are nearly identical with nearly identical standard deviations (Table
192 S23). Furthermore, estimates of the admixture proportion are in agreement with previous estimates
193 of roughly 1% of Neanderthal introgression (Green et al., 2010; Mafessoni et al., 2020; Prüfer et al.,
194 2017; Prüfer et al., 2014).

195 We also sought out to apply our methods in a non-human data set, and we chose canid data
196 to investigate the behavior of ancestral and derived allele sharing site pattern-based introgression

197 metrics when only one individual per population is sequenced. To do so, we calculated all site
198 patterns and introgression metrics from high-coverage whole-genome sequences for the ((Dingo,
199 Basenji), Israeli Wolf) trio where we used the Golden Jackal as an outgroup—we chose this trio as
200 it has the largest significant Z -score for *Patterson's D* reported in the original paper (Freedman et
201 al., 2014). It should be noted that it is highly unlikely that any biological system has experienced
202 a demographic history that mirrors the IUA model which the underlying expectations for site
203 pattern differences are based on; to this extent we propose a definition of *approximately equivalent*,
204 which is defined as site pattern differences having the same sign and same order of magnitude.
205 Notably, even though the demographic history of canids is known to violate the underlying IUA
206 model, the site pattern differences are concordant with our definition of *approximately equivalent*
207 (Table S24). Additionally, concordant with the original findings from *Freedman et al. 2014*, we find
208 that all introgression detection metrics are statistically significant and that all inferred admixture
209 proportions varied at most by 1.4% Israeli Wolf ancestry between quantification metrics (Table
210 S24).

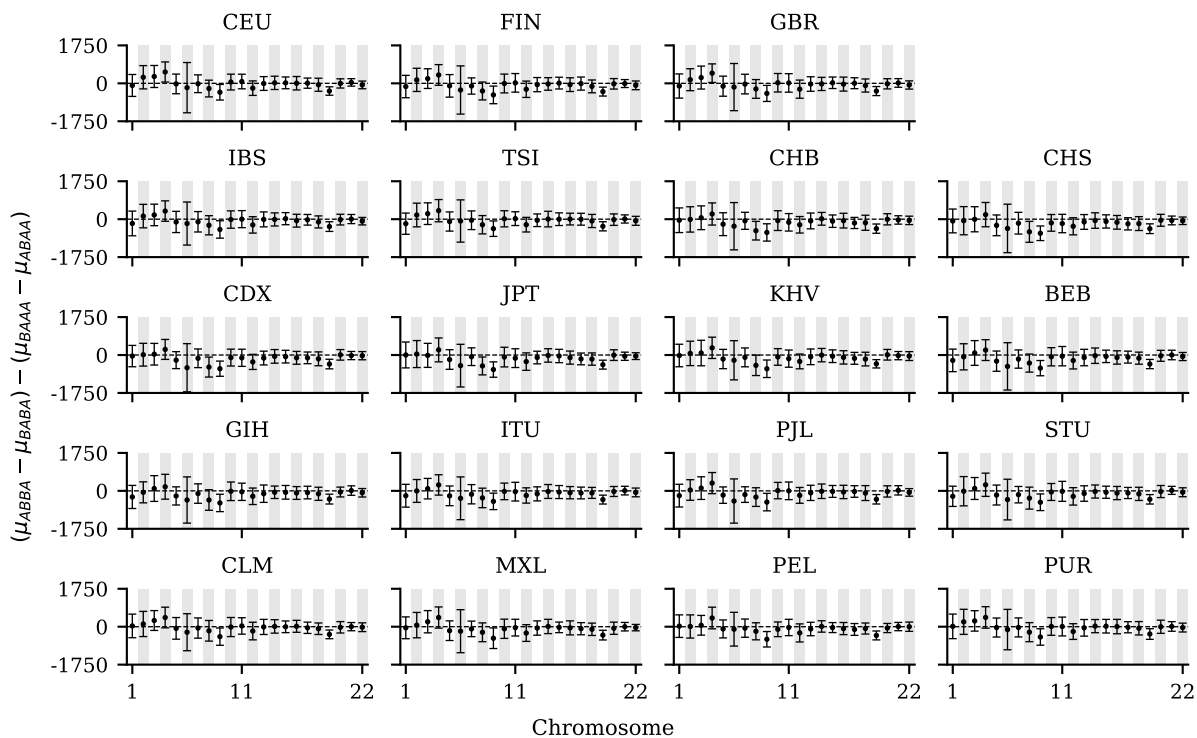


Figure 4: The mean (dots), two standard deviation (error bars), and the theoretical expectation (dashed line and $y = 0$) for the mean difference of site pattern differences among trios per chromosome for every non-African population in the 1000 Genomes Project.

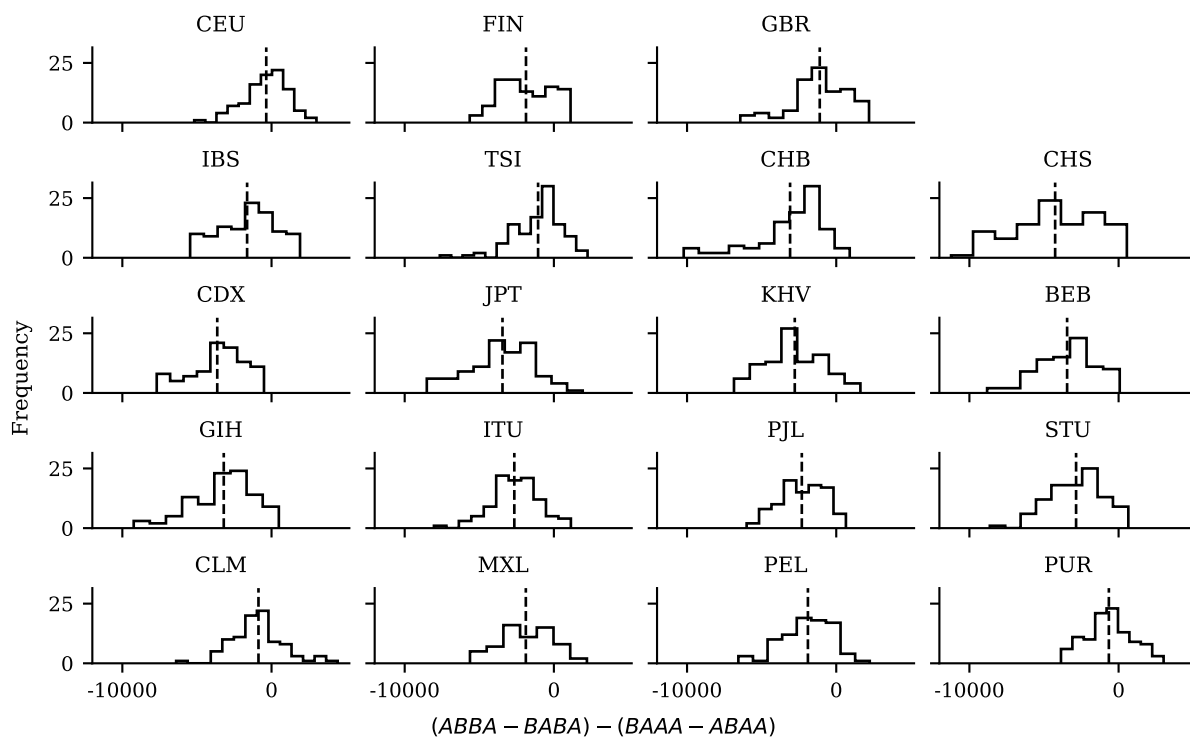


Figure 5: Distributions of difference of site pattern differences among trios for every non-African population in the 1000 Genomes Project. The black dashed line represents the genome-wide mean difference of site pattern differences among trios per non-African population.

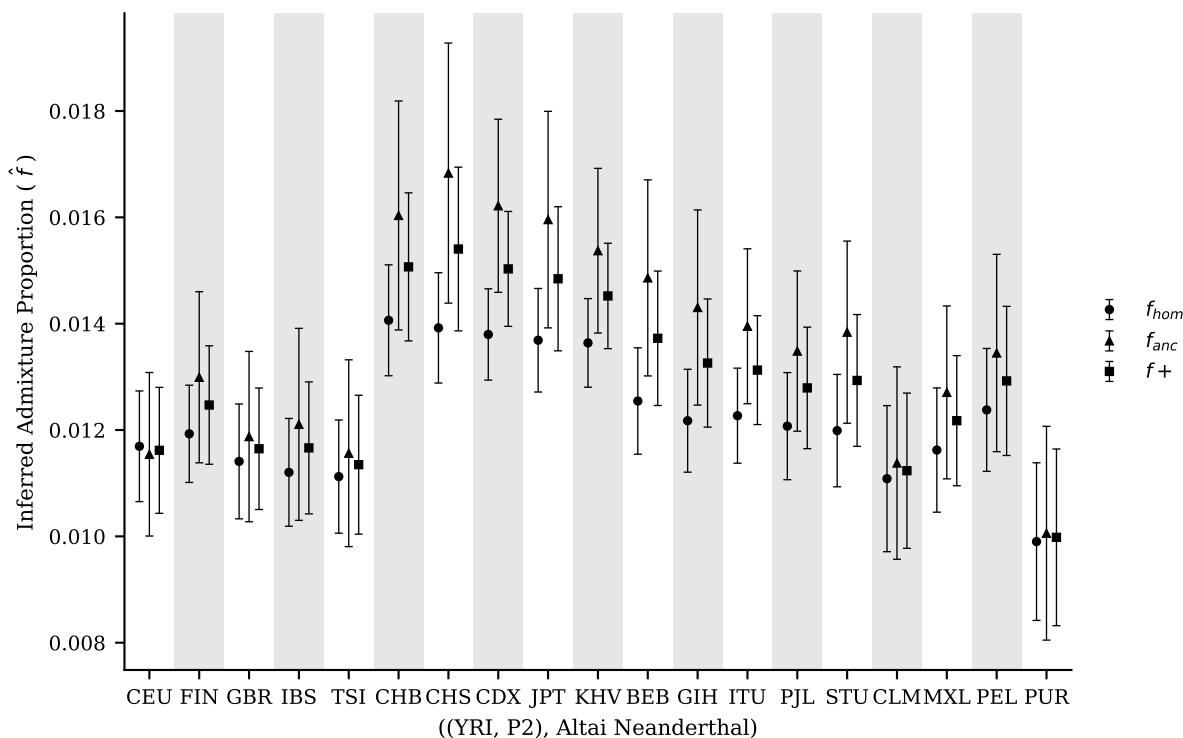


Figure 6: The mean (dots, triangles, and squares) and standard deviation (error bars) of f_{hom} , f_{anc} , and f_+ among trios for every non-African population in the 1000 Genomes Project.

211 4 Discussion

212 The genomic era continues to provide evidence that post-divergence gene flow events are much
 213 more common than previously appreciated. Characterizing what are the effects and consequences
 214 of introgression across the tree of life requires providing robust tools that are able to efficiently
 215 and confidently detect and quantify such events. Here, in addition to using derived allele sharing
 216 between a putative donor and recipient population, we proposed to also incorporate ancestral allele
 217 sharing to construct estimators of the admixture proportion—e.g. f_{anc} and f_+ . In our study we
 218 benchmark D , D_{anc} , D_+ , f_{hom} , f_{anc} , and f_+ which can be used to detect and quantify introgression.
 219 In particular, we use coalescent theory, simulations, and publicly available empirical data, to show
 220 that genome-wide site pattern-based tests of introgression that rely on ancestral, derived, and both
 221 patterns of allele sharing perform comparably, providing researchers the opportunity to corroborate
 222 claims of introgression from multiple metrics that exploit different genomic signals.

223 Our results show that D , D_{anc} , and $D+$ can detect the presence of introgression. On average
224 D_{anc} has less power to detect introgression when compared to D and $D+$, but D_{anc} on average also
225 exhibits the lowest FPR (Tables S7-S9). D tends to slightly have more power to detect introgression
226 than $D+$ but also tends to have higher FPR compared to $D+$ (Tables S7-S9). In empirical data
227 we found that D , D_{anc} , and $D+$ gave concordant results, suggesting that all of the aforementioned
228 statistics are appropriate for detecting introgression on genome-wide data sets. The power to
229 correctly infer the admixture proportion suffers from theoretical constraints. Specifically, f_{hom} ,
230 f_{anc} , and $f+$ all have the same theoretical expectation of $\frac{(f) \cdot (T_{P3} - T_{GF})}{T_{P3} + 2N}$ (see equations A7-A9 in the
231 Appendix), which makes it clear that we will always underestimate the admixture proportion. In
232 simulated data the mean f_{hom} , f_{anc} , and $f+$ values closely mirrored the theoretical expectations
233 (Figure 3; Table S13) and all quantification metrics exhibit nearly identical $RMSE$ and MAE
234 with respect to the simulated admixture proportion (Tables S13-S15). Additionally, in human
235 data not only do we observe nearly identical estimates of the admixture proportions between all
236 metrics studied here, but also nearly identical standard deviations for both trio and population
237 level estimates (Figure 3; Table S23 and S22). Taken together we propose that researchers make
238 use of both derived and ancestral allele sharing site pattern-based tests of introgression to make
239 more thorough assessments about the presence and amount of introgression. To this extent we also
240 developed a lightweight python package RIPTA (**R**e-evaluating **I**ntrogression site **P**atterns **T**hrough
241 **A**ncestral alleles) to calculate all site patterns and introgression metrics studied in here.

242 Our theoretical and simulation results have shown that the expectation of site pattern differences—
243 i.e., $(ABBA - BABA) - (BAAA - ABAA)$ —is zero. This is true even when we consider complex
244 demographic histories (Figure 1). When we count these site patterns using human and archaic
245 genomes, we found that most non-African chromosomes in the TGP do not depart from this theo-
246 retical expectation (Figures 4-5; Table S21). One exception is chromosome 19 (see Figure 4), where
247 the difference of $(BAAA - ABAA)$ is larger than $(ABBA - BABA)$, and this is true across all
248 non-African populations. We tested to see if more complex demographic histories that have been
249 proposed for human populations could lead to deviations from zero. For example, we tried incor-
250 porating population expansions and contractions, multiple pulses of introgression, and a dilution
251 in the recipient population, and none of these models result in a difference that deviates from zero
252 (Tables S5-S6 and S19-S20).

253 Another explanation for this observation, could be a component of evolution that our simulations
254 ignored, such as natural selection. Studies have shown that Neanderthal alleles were originally costly
255 on the modern human genetic background due to genetic load (Harris & Nielsen, 2016; Kim et al.,
256 2018). It has also been shown that the effects of Neanderthal ancestry is negatively correlated with
257 exon density under an additive fitness model (Kim et al., 2018). Given that chromosome 19 has
258 the highest gene density in the genome (Grimwood et al., 2004), we hypothesize that the effects of
259 natural selection may influence these site patterns. However, while some studies have shown that
260 derived archaic alleles were likely mostly deleterious in modern humans, we know very little about
261 what evolutionary forces (if any) acted on ancestral alleles that were re-introduced into modern
262 humans with archaic introgression. We acknowledge that the *BAAA* and *ABAA* site patterns
263 are likely to be more sensitive to differences in mutations rate in the two sister populations—i.e.,
264 *P1* and *P2*—than *ABBA* and *BABA* sites. This might be contributing to observation that the
265 difference of (*BAAA* - *ABAA*) is larger than (*ABBA* - *BABA*), where more *BAAA* sites could
266 indicate a higher mutations rate in *P1* vs *P2*. As this is only observed in chromosome 19, we think
267 it is more likely that other evolutionary forces might be contributing to this signature, but more
268 work is needed to characterize what can lead to deviations from zero.

269 In summary, we have provided further evidence that ancestral allele sharing site patterns are
270 informative for detecting introgression on a genome-wide scale and are robust to realistic models
271 of human demography. Additionally, we have derived novel estimators of the admixture propor-
272 tion and demonstrated that these methods provide reliable estimates of the admixture proportion.
273 Applying it to empirical data in humans suggests that chromosome 19 is an outlier with respect to
274 the difference in ancestral allele sharing site patterns. None of the demographic histories that we
275 simulated lead to deviations, so we hypothesize that other non-neutral processes may be at play.
276 More work to investigate how natural selection acted on ancestral alleles that were reintroduced
277 into human populations through introgression with archaic humans might provide insights into how
278 evolutionary forces have shaped introgressed genetic variation.

279 5 Materials and Methods

280 All annotated code to recreate all analyses and annotated Jupyter Notebooks with walk-
281 throughs of how to perform all analyses described in this paper can be found at <https://github.com/>

282 [David-Peede/anc-der-intro-proj](#). The RIPTA package to calculate site patterns and all introgression
283 metrics studied here can be found at <https://github.com/David-Peede/RIPTA>.

284 5.1 Simulation Design

285 All demographic models were quality controlled using `msprime v1.1.1` and visualized using
286 `demesdraw v0.3.0` (Baumdicker et al., 2022; Gower et al., 2022). All `demes .yaml` files with de-
287 mographic histories needed to reproduce our results can be found [https://github.com/David-Peede/](https://github.com/David-Peede/anc-der-intro-proj/tree/main/simulations/yamls)
288 [anc-der-intro-proj/tree/main/simulations/yamls](https://github.com/David-Peede/anc-der-intro-proj/tree/main/simulations/yamls).

289 5.1.1 IUA Model

290 To assess the statistical power of all introgression metrics discussed in this study we simu-
291 lated whole-genome sequences based on a three-taxon tree following IUA model described in *Du-*
292 *rand et al., 2011*, and in *Green et al., 2010*. Using the coalescent simulator `msprime v1.1.1` we
293 simulated 100 Mb genomes under a demographic model with the following parameters: $T_{P3} =$
294 16,000 generations ago, $T_{P2} = 4,000$ generations ago, $T_{GF} = 1,600$ generations ago, constant
295 and equal $N_e = 10,000$ for all populations, $r = 1e^{-8}$ per bp per generation, and $\mu = 1.5e^{-8}$
296 per bp per generation under a Jukes-Cantor substitution model (Baumdicker et al., 2022). Our
297 parameter space was constructed to reflect a simplified version of human evolution with intro-
298 gression from the Neanderthal population into the Eurasian population as was originally de-
299 scribed in *Racimo et al., 2017*. The divergence times, timing and direction of introgression,
300 and mutation and recombination rates are all direct reflections of the original parameter space,
301 and represent plausible parameter values for the well studied case of Neanderthal introgression
302 (Racimo et al., 2016). The only model parameter varied was the simulated admixture propor-
303 tion $f \in \{0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5\}$ which was used to
304 determine the behavior of the introgression metrics studied here, for both plausible and theoretical
305 amounts of introgression. For each of the 15 admixture proportions, 100 replicate simulations were
306 performed, either $n = 1$ or $n = 100$ simulated genomes were sampled per potential recipient popu-
307 lation per replicate, and one simulated genome was sampled from the donor population, which were
308 then used to calculate site pattern counts and all introgression metrics described in this paper.

309 5.1.2 Non-IUA Models

310 To determine the statistical power of all introgression metrics in a more realistic model of human
311 demographic history, we used the used the demographic model proposed by *Ragsdale and Gravel*
312 *2019* implemented in `stdpopsim v0.1.2` (Adrion et al., 2020; Ragsdale & Gravel, 2019). To make
313 this model more comparable to our analyses using the IUA model we modified the model (see Figure
314 S2) to consist of discrete pulses of introgression—one pulse into the Eurasian population, and one
315 pulse into CEU and CHB populations respectively—by taking the midpoint of the two periods of
316 continuous bidirectional gene flow described in the original model. The simulation design for the
317 modified *Ragsdale and Gravel 2019* model is the exact same as described in the previous section.
318 Additionally, we ran all sets of analyses on the original model (see Figure S3) using the original
319 estimates of bidirectional gene flow (Ragsdale & Gravel, 2019). Lastly, to assess if the difference of
320 site pattern differences would significantly deviate from zero we chose to simulate multiple pulses of
321 introgression, ghost introgression, and a dilution event from a so called "Basal Eurasian" population
322 (see Figures S4-S5). We used demographic parameters described in *Rogers and Bohlender 2015*,
323 *Villanea and Schraiber 2019*, and *Ragsdale and Gravel 2019* to run 100 replicate simulations per
324 all pairwise possibilities of plausible Neanderthal $f_{NEA} \in \{0, 0.005, 0.01, 0.015, 0.02\}$ and Denisovan
325 $f_{DEN} \in \{0, 0.005, 0.01, 0.015, 0.02\}$ admixture proportions for sampling schemes of $n = 1$ and $n =$
326 100 simulated genomes from the potential recipient populations, and one simulated genome from
327 the Neanderthal population (Ragsdale & Gravel, 2019; Rogers & Bohlender, 2015; Villanea &
328 Schraiber, 2019).

329 5.2 Assessing Introgression Metrics In Simulated Data

330 For all 100 replicate simulations per admixture proportion we assessed the theoretical expect-
331 ation that $(ABBA - BABA)$ is equivalent to $(BAAA - ABAA)$ by computing the difference of
332 site pattern differences—i.e., $(ABBA - BABA) - (BAAA - ABAA)$ —per replicate to construct a
333 z -distribution. We then assessed if the absolute value of the mean difference of site patterns dif-
334 ferences significantly differed from the theoretical expectation of zero using the `scipy.stats.sf`
335 function in `scipy v1.7.2`, and corrected for multiple comparisons using the Bonferroni correc-
336 tion (Virtanen et al., 2020). To determine the significance of introgression detection metrics—i.e.,
337 D , D_{anc} , and $D+$ —for each simulation replicate we built a bootstrapped distribution of 1,000

338 bootstrapped replicates, where each bootstrapped replicate consists of concatenating 1,000 100 kb
339 windows that were randomly sampled with replacement to build 100 Mb bootstrapped genomes
340 for each focal population. The bootstrapped genomes were then used to construct z -distributions
341 of D , D_{anc} , and $D+$ per replicate, and a nominal p -value of < 0.05 was used as the significance
342 threshold. The power to detect introgression using D , D_{anc} , and $D+$ was then determined by the
343 proportion of replicates that were statistically significant per admixture proportion. The power
344 to accurately infer the true—i.e., simulated—admixture proportion using f_{hom} , f_{anc} , and $f+$ was
345 assessed by calculating root-mean-square-error ($RMSE$) and mean-absolute-error (MAE) using the
346 following equations:

$$RMSE = \sqrt{\frac{\sum_{i=1}^{100} (\hat{f}_i - f_{true})^2}{100}} \quad (7)$$

$$MAE = \frac{\sum_{i=1}^{100} |\hat{f}_i - f_{true}|}{100} \quad (8)$$

347 where \hat{f}_i represents the inferred admixture proportion for the i^{th} replicate simulation out of 100 total
348 replicates and where f_{true} represents the admixture proportion used in the simulation to infer \hat{f} . We
349 chose to assess the performance of each introgression quantification metric using both the $RMSE$
350 and MAE because both values are in the same units as the admixture proportion and because both
351 quantify the amount of dispersion in the estimates of the inferred admixture proportion around the
352 simulated admixture proportion where the $RMSE$ values gives more weight to inferred estimates
353 that have the large amounts of error while MAE values equally weight the amount of error in all
354 inferred estimates.

355 5.3 Human Application

356 The all sites VCF files for the high-coverage genome of the Altai Neanderthal were downloaded
357 from <https://www.eva.mpg.de/genetics/genome-projects>, the curated and imputed high quality
358 genotypes from the phase three release of the 1000 Genomes Project (TGP) in VCF format was
359 download from <http://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502>, and the ancestral al-
360 lele calls in fasta format for the Hg19 assembly using the Enredo, Pecan, Ortheus (EPO) pipeline
361 was download from http://ftp.ensembl.org/pub/release-74/fasta/ancestral_alleles (1000 Genomes

362 Project Consortium et al., 2015; Herrero et al., 2016; Prüfer et al., 2014). The autosomal TGP
363 and Altai Neanderthal VCF files were merged using the `merge` command from the `bcftools v1.13`
364 package and the resulting merged VCF files were then filtered to only contain sites with no missing
365 data, an ancestral allele call, and bi-allelic SNPs with a mapping quality of 25 or higher and a
366 genotype quality of 40 or higher (Li, 2011). Site patterns and all introgression metrics were then
367 calculated for every non-African individual using the following configuration $P1 = NA18486$ (a
368 randomly chosen individual from Yoruba in Ibadan, Nigeria), $P2 =$ non-African individual, $P3 =$
369 Altai Neanderthal, and $O = EPO$ ancestral allele call. Statistical tests based on the difference
370 of site pattern difference distributions per non-African TGP population were carried out in the
371 same manner as described in previous methods subsection for both individual chromosomes and
372 the entire genome. Next, site patterns and all introgression metrics were additionally calculated
373 from derived allele frequencies using the following configuration $P1 =$ Yoruba in Ibadan, Nigeria
374 (YRI), $P2 =$ non-African population, $P3 =$ Altai Neanderthal, and $O = EPO$ calls. Lastly, for
375 the population level comparisons, the significance of introgression detection metrics—including cor-
376 recting for multiple comparisons using the Bonferroni correction—and standard deviations for the
377 introgression quantification metrics were determined from bootstrapped distributions consisting of
378 1,000 replicates, which were generated from 1,000 bootstrapped genomes per population, where
379 a single bootstrapped genome was constructed from concatenating 303 randomly sampled with
380 replacement 10 Mb windows.

381 5.4 Canid Application

382 The merged VCF files for the high-coverage canid data-set were downloaded from [https://doi.](https://doi.org/10.5061/dryad.sk3p7)
383 [org/10.5061/dryad.sk3p7](https://doi.org/10.5061/dryad.sk3p7) (Freedman et al., 2014). The autosomal VCF files were then filtered by
384 the genomic-feature (`GF=1`) flag using the `view` command from the `bcftools v1.13` package. Ad-
385 ditionally, when performing calculations we only used sites where all focal individuals passed their
386 respective sample filters—i.e., `SF=1` flag. We calculated site patterns and all introgression metrics
387 for the configuration $P1 =$ Dingo, $P2 =$ Basenji, $P3 =$ Israeli Wolf, and $O =$ Golden Jackal
388 because the aforementioned trio had the highest degree of confidence for a significant *Patterson's*
389 *D* reported in the original paper (Freedman et al., 2014). Lastly, the significance of introgres-
390 sion detection metrics and standard deviations for the introgression quantification metrics were

391 determined from bootstrapped distributions consisting of 1,000 replicates, which were generated
392 from 1,000 bootstrapped genomes per individual canid, where a single bootstrapped genome was
393 constructed from concatenating 220 randomly sampled with replacement 10 Mb windows.

394 **6 Data Availability**

395 All annotated code and previously published publicly available data sets needed to reproduce
396 the entirety of this manuscript can be found at the url links described in the methods section.

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